DESIGN AND DEVELOPMENT OF NANOSTRUCTURED LIPID CARRIER CONTAINING COMBINATION OF LINAGLIPTIN AND CANAGLIFLOZIN FOR THE TREATMENT OF DIABETES

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TABLE OF CONTENTS

Sr. No.	Title	Page No.
1	INTRODUCTION	1-6
2	DRUG PROFILE	6-11
3	LITERATURE REVIEW	11-12
4	AIM AND OBJECTIVES	12-13
5	RESEARCH ENVISAGED AND NOVELTY	13-14
6	PLAN OF WORK	14-15
7	REFERENCES	15-18

LIST OF TABLES

Table No.	Title of Tables	Page No.
1	LINAGLIPTIN'S PROFILE	6-9
2	CANAGLIFLOZIN'S PROFILE	9-11

1. INTRODUCTION

1.1 Diabetes

Diabetes mellitus, or simply diabetes, is a set of metabolic illnesses in which a person's blood sugar is elevated due to either insufficient insulin production by the body or a failure of the cells to respond to the insulin that is produced. Polyuria (frequent urination), polydipsia (increased thirst), and polyphagia (increased appetite) are the characteristic symptoms of this elevated blood sugar. Over 100 million people globally (6% of the population) suffer with diabetes mellitus (DM), the most prevalent endocrine condition. It has been shown to harm numerous bodily systems, including the heart, kidney, blood vessels, eyes, and nerves. There are two forms of diabetes mellitus: non-insulin dependent diabetes mellitus (NIDDM, Type II) and insulin dependent diabetes mellitus (IDDM, Type I). In contrast to Type II diabetes, which is defined by peripheral insulin resistance and decreased insulin production, Type I diabetes is an autoimmune illness that is characterized by a local inflammatory response in and around islets, followed by the selective death of cells that secrete insulin. Diabetes mellitus increases the risk of numerous consequences, including peripheral vascular disease, stroke, neuropathy, renal failure, retinopathy, blindness, amputations, and cardiovascular illnesses. The main purposes of drugs are symptom relief and life preservation. Preventing long-term diabetes problems and extending life expectancy by removing risk factors are secondary goals. Patients with type 1 diabetes are treated primarily with insulin replacement medication, whereas type 2 diabetes is treated and managed mostly with dietary and lifestyle changes **(Deshmukh *et al.*, 2015).** With 20–30% of cases, kidney damage is one of the most prevalent side effects of type 1 and type 2 diabetes mellitus (T1DM and T2DM, respectively). Diabetic nephropathy's main effects include increased cardiovascular disease, mortality, and kidney function loss that results in end-stage renal disease (ESRD). In order to effectively treat type 2 diabetes mellitus, dipeptidyl peptidase-4 (DPP-4) inhibitors are a very promising new family of oral medications that can be used in conjunction with existing prescribed antidiabetic medications to meet blood glucose targets. The dose of any DPP-4 inhibitor must be adjusted for all patients with type 2 diabetes mellitus who have compromised renal function, with the exception of linagliptin, for which renal excretion is a minor elimination pathway. Therefore, linagliptin is the medication most commonly used to treat individuals with renal failure who have type 2 diabetes mellitus **(Mikov *et al.*, 2020).**Patients

with type 2 diabetes mellitus and chronic kidney disease can also safely reduce their risk of renal and cardiovascular events by using canagliflozin, an inhibitor of sodium-glucose co-transporter 2 (SGLT2) **(Wada *et al.*, 2022).**

1.1.1 Type 1 Diabetes

The most prevalent chronic autoimmune disease in young patients is type 1 diabetes mellitus (T1DM), which is primarily diagnosed in children and adolescents. It is characterized by the death of pancreatic cells, which causes the body to become insulin deficient and hyperglycemic, exhibiting the "classic" trio of symptoms: polydipsia, polyphagia, and polyuria. Patients with type 1 diabetes require daily insulin injections because of the complete lack of endogenous insulin resulting from the autoimmune destruction of pancreatic cells, which necessitates the prompt provision of exogenous insulin. This therapy is a lifelong practice. The causes of these autoimmune reactions, which are sometimes referred to as "environmental factors" that contribute to the development of the disease, are yet unknown **(Rodrigues Oliveira *et al.*, 2023).**

1.1.2 Type 2 Diabetes

Type 2 Diabetes is one of the most common metabolic disorders worldwide and its development is primarily caused by a combination of two main factors: defective insulin secretion by pancreatic β-cells and the inability of insulin-sensitive tissues to respond to insulin. According to the World Health Organization (WHO) diabetes mellitus is a chronic, metabolic disease characterized by elevated levels of blood glucose, which leads over time to damage to the heart, vasculature, eyes, kidneys and nerves. Over 90% of diabetes mellitus cases are T2DM, a condition marked by deficient insulin secretion by pancreatic islet β-cells, tissue insulin resistance (IR) and an inadequate compensatory insulin secretory response. Progression of the disease makes insulin secretion unable to maintain glucose homeostasis, producing hyperglycemia **(Galicia-Garcia *et al.*, 2020).**

1.2 Nanostructured lipid carrier

Nanostructured lipid carrier (NLCs) are the second generation of solid lipid nanoparticles (SLNs), composed of a binary mixture of solid lipid and liquid lipid, the average size in the range of 10–500 nm. The solid lipid is mixed with the liquid lipids preferably in a ratio of 70:30

up to 99.9:0.1. They contain a special nanostructure, which results in the improvement in drug loading as well as firm incorporation of the drug inside increasing the storage time. The NLCs can be administered to the patients using oral, topical, intravenous (IV), as well as ocular routes **(Shamama *et al*., 2022).**

1.2.1 Role of Nanostructured lipid carrier in different delivery system

Nanostructured lipid carriers are biocompatible delivery systems for a variety of pharmacological, cosmetic, and biochemical substances. Over the past ten years, several medications or active ingredients have been trapped in hydrophilic, lipophilic, and labile molecules such proteins and peptides that are nanostructured lipid carriers. Their preparation using physiologically acceptable lipids is their primary characteristic. These carriers are very well suited for various applications, such as topical, oral, parenteral, inhalational, and ophthalmic administration, due to the large variety of surfactants and co-surfactants that may be used to produce such particles **(Iqbal *et al*., 2012).**

1.2.2 Advantages of Nanostructured lipid carriers (NLCs) (Jaiswal *et al*., 2016)

- Better physical stability
- Ease of preparation and scale-up
- Increased dispersability in an aqueous medium
- High entrapment of lipophilic drugs and hydrophilic drugs
- Controlled particle size
- An advanced and efficient carrier system in particular for lipophilic substances
- Increase of skin occlusion
- Extended release of the drug
- One of the carriers of choice for topically applied drugs because their lipid components have an approved status or are excipients used in commercially available topical cosmetic or pharmaceutical preparations
- Small size of the lipid particles ensures close contact to the stratum corneum thus enhancing drug penetration into the mucosa or skin

- Improve benefit/risk ratio
- Increase of skin hydration and elasticity
- These carriers are highly efficient systems due to their solid lipid matrices, which are also generally recognized as safe or have a regulatory accepted status

1.2.3 Limitations with lipid nanoparticles

Despite the great potential of NLCs in targeted delivery, they face certain limitations like:

- Cytotoxic effects related to the nature of matrix and concentration,
- Irritative and sensitising action of some surfactants,
- Application and efficiency in case of protein and peptide drugs and gene delivery systems still need to be better exploited
- Lack of sufficient preclinical and clinical studies with these nanoparticles in case of bone repair.

1.2.4 Methods for preparation of Nanostructured lipid carriers (Gomaa *et al*., 2022)

Several techniques are reported in the literature for the preparation of Nanostructured lipid carriers. These include high pressure homogenization, microemulsion, probe sonication, solvent diffusion, solvent emulsification evaporation, solvent injection/solvent displacement, and phase inversion.

1.2.4.1 High pressure homogenization

For thermostable medications, this technique uses hot, high-pressure homogenization (HPH); for thermosensitive medications, it uses cold HPH. Temperatures higher than the lipids' melting points are used for hot homogenization. The two phases are created independently: the lipid phase, which consists of liquid and solid lipids and lipophilic emulsifiers, and the aqueous phase, which consists of hydrophilic emulsifiers and double-distilled water. Prior to mixing, the two phases are heated to a high temperature individually. A high-shear homogenizer can be used to homogenize the mixture, and additional sonication can be used to achieve a tiny and consistent size distribution.

Cold HPH entails combining the drug and lipid phase at a temperature that is marginally above the lipid melting point. The combination is then promptly cooled with liquid nitrogen or dry ice to enable the solid lipid particles to recrystallize quickly. In order to create smaller nanostructures, the particles are further ground down and emulsified in a cold aqueous phase using a high-pressure homogenizer. Large-scale production, avoiding organic solvents, and enhanced product stability are some benefits of this process, albeit it may be difficult to meet the high pressure and temperature requirements. Furthermore, rather than creating a uniform size distribution, insufficient homogeneity could lead to the presence of microparticles.

1.2.4.2 Microemulsion

This technique involves combining melted fats with a hydrophilic aqueous phase that contains a surfactant and a co-surfactant to create an emulsion that can be either w/o or o/w, depending on the ratios utilized. The particles in the emulsion are then broken down into the micron size range by vigorously mixing them. Following the formation of a transparent, thermodynamically stable microemulsion, it is further dispersed in a cold hydrophilic phase to induce NLCs and further reduce particle size. This process is easy to use, affordable, repeatable, appropriate for thermolabile medications, and it doesn't require any extra energy or equipment to produce NLCs. However, this technique's primary drawback is thought to be the substantial amounts of surfactants that must be used.

1.2.4.3 Solvent injection method

Solvent injection method is a simple, fast production technique that involves dissolving of lipids in a water-miscible solvent followed by a quick injection into an aqueous solution of surfactants through an injection needle. The benefits of this approach include minimal preparation, avoidance of high temperatures, shear stress, and expensive equipment. This method's primary drawbacks, however, are its low particle concentration and usage of organic solvents.

1.2.4.4 Phase inversion

This approach involves gently mixing the drug, lipid, water, and surfactant, then heating the mixture to a temperature higher than the surfactant's phase inversion temperature. The surfactant undergoes dehydration during the heating process (above the inversion temperature), which

alters its hydrophilic lipophilic balance and, in turn, its affinity for each phase, i.e., the inverted emulsion. Rapid cooling (such as with an ice bath) causes the surfactant to revert to its hydrophilic state, which permits the production of tiny NLC particles. This method's low energy input and avoidance of organic solvents are its advantages. However, produced NLCs may have low stabilities and occasionally require multiple temperature cycles.

1.2.4.5 Emulsification-solvent evaporation

This technique was initially created in 1979. This technique of polymer emulsification enables the creation of polymeric particles. Using this procedure, lipids and medications are dissolved in an organic solvent that is water immiscible (such as cyclohexane, dichloromethane, toluene, or chloroform), and then they are emulsified in an aqueous phase that contains the surfactant before the solvent evaporates. The pre-emulsion that has been created is sonicated. To obtain the aqueous NLC dispersions, the dispersions are then allowed to cool to room temperature. This technique is linked to a quick and simple manufacturing procedure. However, the suspension that is created is diluted and requires additional evaporation or ultra-filtration, and it also uses organic solvents.

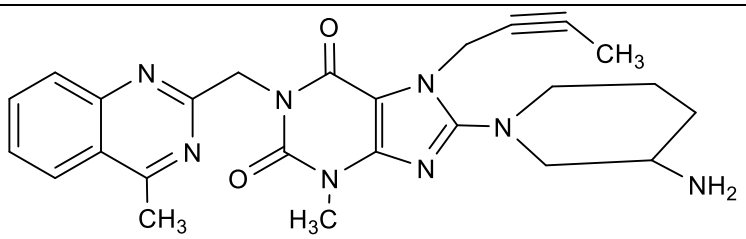
2. Drug profile

2.1 Linagliptin

Linagliptin is a dipeptidyl peptidase-4 (DPP-4) inhibitor used to manage hyperglycemia in patients with type 2 diabetes mellitus.

Table 1: Linagliptin's Profile (Graefe- Mody *et al.*, 2012), (Ceriello & Inagaki 2017), (Scheen 2013), (Neumiller & Setter 2012), (Veni & Gupta 2020)

Drug synonym	Ondero
IUPAC name	8-[(3*R*)-3-Aminopiperidin-1-yl]-7-but-2-ynyl-3-methyl-1-[(4-methylquinazolin-2-yl)methyl] purine-2,6-dione
Chemical formula	$C_{25}H_{28}N_8O_2$

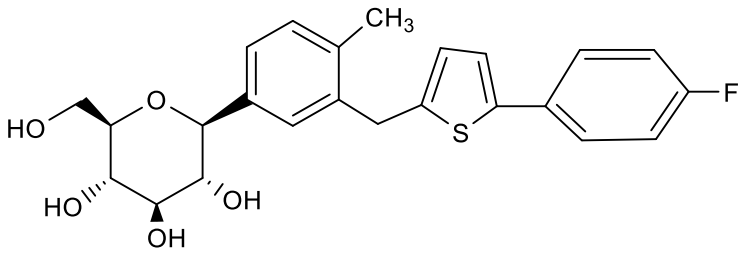
Chemical structure	
Molecular Weight	472.54 g/mol
Solubility	Very slightly soluble in water (0.9 mg/mL). Linagliptin is soluble in methanol (60 mg/mL), sparingly soluble in ethanol.
Melting Point	202-206°C
Indication	In addition to dietary modifications and physical exercise, linagliptin is advised for the management of type II diabetes. It is not recommended for usage in diabetic ketoacidosis or for the management of type I diabetes. In January 2020, the FDA approved a long-acting combination of metformin, linagliptin, and empagliflozin. When used in conjunction with diet and exercise, this medicine has been approved to improve blood sugar management in persons with type 2 diabetes.
Pharmacodynamic	When a 5 mg pill is taken orally, DPP-4 activity is reduced by approximately 80% for a minimum of 24 hours. Reducing DPP-4 activity raises glucagon-like peptide 1 (GLP-1) levels, which lowers HbA1c and blood sugar.
Mechanism of action	Linagliptin is a competitive, reversible DPP (Dipeptidyl peptidase)-4 inhibitor specifically designed for extended inhibition of the DPP-4 enzyme. Linagliptin inhibit the enzymatic degradation of endogenous GLP-1(Glucagon like peptide-1) and GIP(Gastric inhibitory polypeptide) by the enzyme DPP-4. This enzymatic inhibition in turn potentiates the effects of endogenous

	incretin hormones, resulting in enhanced glucose-dependent insulin secretion and suppression of postprandial glucagon secretion from cells of the pancreas. By prolonging the activity of endogenous incretin hormones, DPP-4 inhibitors regulate glucose levels in a glucose-dependent manner.
Absorption	Oral bioavailability of linagliptin is 30%
Volume of distribution	A distribution volume of 1110L is produced by a single injection of 5 mg. A continuous IV infusion of 0.5–10 mg, on the other hand, results in a distribution volume of 380–1540 L.
Protein binding	Linagliptin is 99% protein bound at a concentration of 1nmol/L and 75-89% protein bound at a concentration of >30nmol/L
Metabolism	When linagliptin is given orally, the majority of the medication is removed through feces, with 90% of the dose being excreted undisturbed in both. The predominant linagliptin form detected in blood is CD1790, while M 489 is the predominant form remaining after its elimination. Processes such as oxidation, oxidative breakdown, *N*-acetylation, glucuronidation, and the creation of adducts with cysteine result in the creation of other forms of linagliptin. Mass spectrometry has been used to identify a few of these metabolites, but their structures are yet unknown. The body uses enzymes such as cytochrome P450 3A4, aldo-keto reductases, and carbonyl reductases to break down linagliptin.
Route of elimination	84.7% of linagliptin is eliminated in the feces and 5.4% is eliminated in the urine
Half life	The terminal half-life of linagliptin is more than 100 hours

2.2 Canagliflozin (Joshi *et.*, 2019)

Canagliflozin is a sodium-glucose co-transporter 2 (SGLT2) inhibitor used to manage hyperglycemia in type 2 diabetes mellitus (DM). Also used to reduce the risk of major cardiovascular events in patients with established cardiovascular disease and type 2 DM.

Table 2: Canagliflozin's Profile

Drug synonyms	Invokana Anhydrous Canagliflozin
IUPAC name	2-[3-[[5-(4-Fluorophenyl)thiophen-2-yl]methyl]-4-methylphenyl]-6-(hydroxymethyl)oxane-3,4,5-triol
Chemical formula	$C_{24}H_{25}FO_5S$
Chemical structure	
Molecular Weight	444.517g/mol
Solubility	Canagliflozin is soluble in methanol and buffer medium
Melting Point	90-95 °C
Indication	When taken in conjunction with a nutritious diet and regular exercise, this drug helps persons with type 2 diabetes better control their blood sugar levels. It is also recommended for the prevention of major cardiac problems (heart attacks, strokes, or heart-related deaths) in people with type 2 diabetes as well as the reduction of hospitalization for heart failure in those same people.

Pharmacodynamic	This drug decreases the kidney's glucose threshold (RTG) in a manner that is dose-dependent and increases the quantity of glucose expelled through urine. The lowest blood glucose level at which glucose is detectable in urine is known as the kidney's threshold. After using canagliflozin, the amount of glucose expelled through urine increases while the amount absorbed by the kidneys decreases. This results in a drop in blood glucose levels and improved blood sugar regulation.
Mechanism of action	The kidney's innermost tubules contain the sodium-glucose co-transporter 2 (SGLT2), which reabsorbs glucose that has been filtered out of the fluid inside the tubules. Canagliflozin inhibits the SGLT2 co-transporter's ability to function. This decreases the kidney's glucose threshold (RTG) and lessens the quantity of glucose that is reabsorbed back into the body, causing more glucose to be expelled in the urine.
Absorption	Approximately 65% of canagliflozin is absorbed through the mouth on average. The drug takes four to five days to reach its peak levels in the body when taken daily at doses ranging from 100 mg to 300 mg.
Volume of distribution	The body's distribution of this medicine is extensive. It was found that in healthy persons, the average volume of distribution of canagliflozin in the body upon reaching a steady state following a single injection was 83.5 liters.
Protein binding	Canagliflozin is mainly bound to albumin. The plasma protein binding of this drug is 99%.
Metabolism	*O*-glucuronidation is the main process that breaks down canagliflozin. The UGT1A9 and UGT2B4 enzymes are mainly responsible for its breakdown into two inactive O-glucuronide

	byproducts. In humans, the amount of canagliflozin that is broken down by the cytochrome P450 enzyme CYP3A4 in the liver is relatively modest (less than 7%).
Route of elimination	The quantities of canagliflozin or its metabolites in the urine and feces of healthy persons were measured after they received a single oral dosage of radiolabeled medication.
Half life	11.8(10-13) hours. The apparent terminal half life ($t^{1/2)}$ is 10.6 hours and 13.1 hours for the 100mg and 300 mg doses, respectively.

3. LITERATURE REVIEW

Hammad *et al.*, (2024) studied about a block copolymer structure of chitosan and PVP with uniformly spaced nano-channels was designed to deliver two hypoglycemic drugs effectively and simultaneously. Linigliptin has a non-linear pharmacokinetic profile and 30% oral bioavailability due to its limited permeability. To improve the medications' physicochemical characteristics, cubosomes were altered to function as efficient microdomains. The modified LECF enhanced empagliflozin absorption while maintaining linagliptin efflux, reducing concerns about its non-linear plasma profile.

Yang *et al.*, (2024) studied about Diabetic cardiomyopathy (DCM) which was a major cause of mortality in diabetic populations, and current treatments were insufficient. Canagliflozin, a drug with unknown molecular processes, has been shown to be a potential cardioprotective medication in diabetes. In vitro and in vivo studies showed that canagliflozin reduced heart dysfunction and enhanced mitochondrial function. It increased ATP levels, maintained mitochondrial respiratory capacity, and promoted PINK1-Parkin-dependent mitophagy. However, canagliflozin's protective effects on mitochondrial integrity were neutralized by PINK1 knockdown, suggesting it could be a useful treatment for DCM.

Kenechukwu *et al.*, (2022) developed NLC to improve oral bioavailability and efficacy as metformin hydrochloride (MTH) has been linked to poor absorption, low bioavailability, short

half-life, and high dosage and dose-related side effects, surface-modified. These NLCs were prepared by fusion of beeswax, Phospholipon® 90H, and Capryol-PGE 860, and PEGylated to give 10, 20, and 40% PEGylated lipid matrices. The NLCs showed higher encapsulation efficiencies and loading capacities than the marketed MTH formulation, enhanced pharmacokinetic properties, and sustained blood glucose lowering effect.

Pandey *et al.*, (2020) formulated repaglinide-loaded nanostructured lipid carrier-gel (RG-NLCs-gel) system to improve oral bioavailability of Repaglinide, an effective anti-diabetic drug. The system was optimized for sustained transdermal delivery using a Box-Behnken design. The optimized batch showed sustained release up to 24 hours and two times improvement in bioavailability compared to the marketed oral tablet in a rat model. This suggests the system's potential for other BCS class II drugs.

Neal *et al.*, (2017) study involving patients with type 2 diabetes and significant cardiovascular risk found that canagliflozin, a sodium-glucose co-transporter 2 inhibitor, lowers blood pressure, body weight, albuminuria, and glycemia. The results showed a lower rate of primary outcomes compared to a placebo, but a potential benefit in reducing albuminuria progression and glomerular filtration rate. However, renal outcomes were not statistically significant. Patients treated with canagliflozin had a lower risk of cardiovascular events than those receiving a placebo, but a larger chance of amputation, especially at the toe or metatarsal level.

Plosker *et al.*, (2014) investigated that Canagliflozin was an oral sodium-glucose co-transporter-2 inhibitor used to treat type 2 diabetes. It lowers renal glucose reabsorption by blocking the kidney's SGLT2 transporter protein, lowering blood glucose levels. Canagliflozin improves glycemic control in patients with type 2 diabetes when used as monotherapy or as an adjuvant therapy to metformin and other anti hyperglycemic agents. It has shown positive impacts on other endpoints, such as bodyweight and fasting plasma glucose levels. Side effects include urinary tract infections, increased urination, and female genital mycotic infections. Canagliflozin has mild effects on blood lipid profiles and blood pressure.

4. AIM AND OBJECTIVES

4.1 Aim:- Design and Development of Nanostructured Lipid Carried containing combination of Linagliptin and Canagliflozin for the treatment of Diabetes.

4.2 Objectives:-

- Formulation of an Effective Nanostructured Lipid Carrier System
- Optimize Drug Loading and Encapsulation Efficiency
- To assess the release profile of linagliptin and canagliflozin from the NLC formulation.

5. RESEARCH ENVISAGED AND NOVELTY

Developing a formulation of nanostructured lipid carriers (NLC) that combines canagliflozin and linagliptin is a calculated move to solve a number of important issues with these anti diabetic medications' oral distribution. Both canagliflozin, an inhibitor of sodium-glucose cotransporter-2 (SGLT2), and linagliptin, an inhibitor of dipeptidyl peptidase-4 (DPP-4) are useful in the management of diabetes mellitus because of their complementary mechanisms of action, which increase glucose excretion and enhance insulin secretion, respectively **(Fushimi *et al.*, 2021).** Combination of DPP-4 inhibitor and SGLT2 inhibitor exerts more beneficial effects on β-cell mass and function. These medications have considerable challenges with their pharmacokinetics and tolerability despite their therapeutic benefits **(Fathy Elhabal *et al*., 2023).**

Their low bioavailability and solubility are among the main problems, because linagliptin is poorly soluble in water, oral administration may result in reduced absorption and reduced efficacy. Analogously, canagliflozin's poor oral bioavailability makes its therapeutic application even more challenging.

An advanced remedy for these issues is provided by nanostructured lipid carriers (NLCs) **(Shah *et al*., 2021).** The NLC system offers a number of benefits since it consist of a liquid lipid shell around a solid lipid core. First off, by encasing poorly water-soluble medications within a lipid matrix, NLCs improve their solubility. The improved absorption and improved drug solubility provided by NLCs' nanoscale size increase the bioavailability of canagliflozin and linagliptin. With lower dose of the medications, better solubility and absorption can contribute to more successful glycemic control.

Additionally, they offer the encapsulated medications a regulated and prolonged release. This controlled release mechanism lowers the frequency of dose and may enhance patient compliance

by helping to maintain therapeutic medication levels for a longer amount of time. The NLC formulation can maximize linagliptin and canagliflozin's therapeutic efficacy while reducing drug level fluctuations that may cause side effects or diminished efficacy **(Ilyas *et al.*, 2022).**

They also have the benefit of lessening gastric discomfort. Drugs can be shielded from direct gastrointestinal tract contact by being encapsulated in lipid carriers, which can reduce discomfort and related side effects. By making the treatment plan more bearable for the patients, this protection can improve therapy adherence even further.

NLCs not only offer these advantages but also enhance medication stability. Linagliptin and canagliflozin are protected from environmental conditions that could cause deterioration by the lipid matrix, which helps prolong their shelf life. For the combination therapy to remain effective and intact, this stability is essential **(Ilyas *et al*., 2022).**

6. PLAN OF WORK

6.1 Review of literature

6.2 Procurement of drugs and excipients

6.1 Preformulation studies of drugs

- HPLC method validation
- UV method validation
- Melting point determination
- Solubility studies
- Partition coefficient
- XRD
- FTIR spectra
- DSC

6.2 Preparation of Nanostructured lipid carrier containing combination of Linagliptin and Canagliflozin

6.3 Screening of parameter for design of experiment

6.4 Preparation of Nanostructured lipid as per Design of Experiment

6.5 Lyophilization of Nanostructured lipid carrier

6.6 Evaluation of Nanostructured lipid carrier

- FTIR
- DSC
- XRD
- Percentage yield
- Percentage Encapsulation efficiency
- Percentage drug loading
- TEM
- *In vitro* drug release studies
- *In vitro* drug release kinetics studies

6.7 Stability Studies

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